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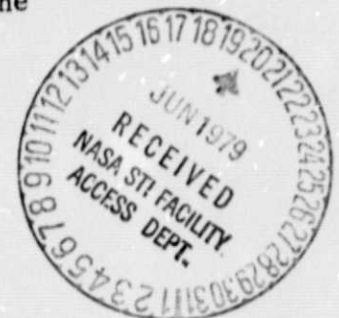
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SUBJECT Study Report on
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This report briefly discusses some of the pros and cons of three methods to study gastrointestinal calcium absorption. These methods are: 1) a balance study, 2) a single isotope method, and 3) a double isotope method. A procedure for the double isotope method is also included.



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Attachment

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A DOUBLE ISOTOPE METHOD OF CALCIUM ABSORPTION

A subject of rising interest in the study of calcium metabolism is the question of whether or not ingested calcium is absorbed by the gastrointestinal tract (GIT) during weightless conditions. It has been suggested that as the duration in space increases the absorption of calcium from the GIT decreases.⁽¹⁾ Measurements of calcium absorption in man can be done in one of three ways: balance studies, single isotope tracer studies, or double isotope methods. Balance studies are frequently used, but are complicated, demanding, and involve assumptions which may not be true or may be hard to maintain under zero-g conditions. These assumptions include a steady-state condition, a constant calcium load in the gut, and that activity, hypothesized to be occurring in the bone, is correct. Tracer methods attempt to alleviate some of the problems typical of balance studies.

Single isotopic methods can be done in one of three ways: tracer injection,⁽²⁻⁵⁾ tracer ingestion,^(6,7) or both consecutively.⁽⁸⁾ When the tracer is injected into the body, an internal calcium standard is established. The calcium standard is compared to stable calcium levels in the blood and urine. This demands that a precise knowledge of the amount of stable calcium being ingested be known. Levels of the tracer in the feces will yield endogenous calcium secretion rates. If the tracer is administered orally the intake of stable calcium is not important, but a standard value of plasma and urinary calcium values must be presumed. A problem typical of either single isotope method is that it must also include a balance study, thereby making it subject to the assumptions typical of balance studies also. The problem is reduced when the two routes of administration are studied in succession. In this case, the biochemical and environmental conditions affecting each study must be assumed to be identical. This assumption can be eliminated though by the use of a double isotope method.

With the double isotope method a plasma calcium standard and a simple, precise quantity of oral calcium are established.⁽⁹⁻¹²⁾ The absorption of calcium

is based upon the ratio of the two tracers found in the blood and the urine. An index of endogenous calcium secretion can also be derived from the ratio of injected tracer in the feces. The technique does not depend upon metabolic balance assumptions nor is it subject to the problems typical of balance assumptions. Calcium bone metabolism will not distort the results because stable calcium levels exert no effect. Both tracers are assumed to have identical metabolic characteristics once in the plasma. The results will yield values in terms of fractional absorption of the oral tracer of calcium. If total calcium absorption rates are desired, total calcium ingested must be known, but the total intake of calcium is not essential to the procedure. In a study of weightlessness involving comparisons of pre- and inflight results, calcium intake must follow similar patterns, but again, the calcium intake value is not important, only that the diets are consistent. The procedure is easy and involves the use of ^{45}Ca and ^{47}Ca . Stable calcium isotopes, not abundantly found in nature, such as ^{46}Ca and ^{48}Ca might be used to replace ^{45}Ca and ^{47}Ca , respectively. (13-15)

Further evaluation of such a technique must be explored.

Procedure:

A seven-day dietary menu will be imposed upon the subjects for the purpose of maintaining a consistent nutritional intake. The menu will rotate so that every 7th day the same foods in the same quantities of each food item will be completely consumed. The diet will begin a week prior to the start of the study and will terminate the final day of the study. The preflight phase will begin one or two weeks prior to the launch, the inflight phase on the same day as or the day following launch, and the postflight phase on the first day of return. Each phase will continue for a total of 7 days.

Each study will begin with an overnight, 12-hour fast. The fast will be terminated with a breakfast consisting of a 10g calcium gluconate syrup and 500 mg of a precisely weighed quantity of polyethylene glycol (PEL, Carbowax 4000) as an inert, unabsorbed, fecal marker. The calcium gluconate syrup

will contain 169 mg of calcium and $10\mu\text{Ci}$ of ^{47}Ca . Two hours later an intravenous dose of $10\mu\text{Ci}$ ^{45}Ca as CaCl_2 in isotonic saline will be administered.

The data will be derived from urinary and plasma samples. Plasma will be collected in 5 ml volumes at 30 minutes, and 12 and 24 hours after injection and every 24 hours thereafter for the next 5 days. A 15 ml sample will be collected from each urinary voiding the day of injection and from the 24-hour pool each succeeding day for the next 6 days. Stool samples of 15 mls will be collected from 4-day pools for the analysis of endogenous calcium secretion.

Measurement procedures involve the quantitation of ^{47}Ca and ^{45}Ca specific activities. ^{47}Ca will be measured with a gamma ray spectrometer at a radiation above 0.8 meV to exclude radiation from ^{47}Sc , a decay product of ^{47}Ca . ^{45}Ca measurements, as a dry calcium oxalate powder, will be made in a micromil end-window gas-flow counter, three months after collection of the sample. The storage of ^{45}Ca before measurement is to allow for the decay of ^{47}Ca and ^{47}Sc to occur. Matched volumes of appropriately diluted standards will be prepared at the time of administration and measured concurrently with the samples. The standards will consist of 0.2 mg of stable calcium as CaCl_2 and a known fraction of ^{47}Ca or ^{45}Ca . The standards of ^{47}Ca must also be controlled for contaminating levels of ^{45}Ca . Commercial ^{47}Ca contains small amounts of ^{45}Ca . The ratio of ^{45}Ca in ^{47}Ca needs to be identified and the correction applied to the fraction of ^{47}Ca absorbed. This assumes that ^{45}Ca and ^{47}Ca are equally absorbed by the GIT.

The basic assumption of this study is that ^{45}Ca and ^{47}Ca share the same kinetic properties once in the body fluids. The decay product, ^{47}Sc , may not share those properties so that its ratio to calcium in the body fluids may vary from that of the administered dose. To standardize the ^{47}Sc the calcium in the samples and standards shortly before counting are precipitated as calcium

oxalate, thereby removing all ^{47}Sc from both materials. All ^{47}Sc subsequent to that is a result of radioactive decay in identical proportions between samples and standards.

The typical procedure of calcium precipitation from the samples is as follows: the sample is brought to a pH of 4.5 and 2 gm of ammonium oxalate are added. One hour later the precipitate of calcium oxalate is collected and ashed at 500°C . The ash is dissolved in 1 ml of concentrated HCl. All of the samples and standards are then diluted to 10 mls with a pH of 4.5. (Two) 2 mls of a 3% solution of ammonium oxalate are added, the precipitate is collected, and dissolved in a few drops of 6 N HCl. The solution is quantitatively transferred to counting vials, dried at 90°C , and prepared for radioactive measurements.

The excretion of calcium into the gastrointestinal tract is considered to be a minor path of elimination. Under zero-g conditions, the influence of this excretory path can be studied via the analysis of ^{45}Ca levels in the feces. Since ^{45}Ca is administered intravenously, biliary secretion is its only form of entrance into the GIT. Fecal samples will be subject to ^{45}Ca determinations and if a check is desired, ^{47}Ca , for unabsorbed doses, corrected by the ^{45}Ca fractional values.

All of the calculations will be in terms of percent fractional administered dose. Fractional absorption will be considered the ratio between the percent fractional oral dose and percent fractional intravenous dose. Fractional endogenous secretion will be considered the percent fractional ^{45}Ca found in the feces. A graph of urinary and serum, fractional absorption values and fecal secretion values will be drawn versus time. The pre-, in-, and postflight graphs will be compared for an evaluation of gastrointestinal calcium absorption and endogenous secretion.

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